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EXAMINER

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Please find below and/or attached an Office communication concerning this application or proceeding.



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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Paper No. 20030806

Application Number: 09/783,338
Filing Date: February 14, 2001
Appellant(s): GLAZER ET AL.

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Todd S. Hofmeister
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed June 20, 2003.

(1) *Real Party in Interest*

A statement identifying the real party in interest is contained in the brief.

(2) *Related Appeals and Interferences*

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) *Status of Claims*

The statement of the status of the claims contained in the brief is correct.

(4) *Status of Amendments After Final*

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) *Summary of Invention*

The summary of invention contained in the brief is correct.

(6) *Issues*

The appellant's statement of the issues in the brief is correct.

(7) *Grouping of Claims*

Appellant's brief includes a statement that claims 6-14 do not stand or fall together and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8).

(8) *Claims Appealed*

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

Uhlmann et al. "Antisense oligonucleotides: a new therapeutic principle" Chemical Reviews, vol. 90 (1990), pp. 544-584.

Mirabelli et al. "In vitro and in vivo pharmacologic activities of antisense oligonucleotides" Anticancer Drug Design, vol. 6 (Dec 1991), pp. 647-661.

Puri et al. "Targeted gene knockout by 2'-O-aminoethyl modified triplex forming oligonucleotides", J. Biol. Chem. vol. 276, no. 31 (2001), pp. 28991-28998.

Lin et al. "Stability of DNA triplexes on shuttle vector plasmids in the replication pool in Mammalian cells" J. Biol. Chem. vol. 275, no. 50, (2000) pp. 39117-39124.

Vasquez et al. "Chromosomal mutations induced by triplex-forming oligonucleotides in mammalian cells" Nucleic Acids Research, vol. 27, no. 4 (1999) pp. 1176-1181.

Chan et al. "Targeted Correction of an Episomal Gene in Mammalian Cells by a Short DNA Fragment Tethered to a Triplex-forming Oligonucleotide" Journal of Biological Chemistry, vol. 274, no. 17 (1999) pp. 11541-11548.

Barre et al. "Unambiguous demonstration of triple-helix-directed gene modification" Proceedings of the National Academy of Sciences, vol. 97, no. 7 (2000) pp. 3084-3088.

Wang et al. "Targeted Mutagenesis in Mammalian Cells Mediated by Intracellular Triple Helix Formation" Mol. Cell. Biol. vol. 15, no. 3 (1995) pp. 1759-1768.

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 6-14 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for in vitro site directed mutagenesis of a target DNA molecule or site directed mutagenesis of a target DNA molecule ex vivo in cultured or isolated cells, but does not reasonably provide enablement for in vivo methods of site directed mutagenesis of a target DNA molecule. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention and breadth of claims

Claims 6-14 are broadly drawn to methods of site directed mutagenesis comprising a mutagen incorporated into single stranded nucleic acid that forms a triple

helix with the target region which encompasses in vivo, ex vivo and in vitro methods. In fact the specification recites that the present invention provides in vivo and in vitro site directed mutagenesis of a target DNA molecule. However, as will be further discussed, there is no support in the specification and prior art for the in vivo methods, only for ex vivo or in vitro methods. The invention is an class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." *Mycogen Plant Sci., Inc. v. Monsanto Co.*, 243 F.3d 1316, 1330 (Fed. Cir. 2001).

The unpredictability of the art and the state of the prior art

The specification recites site specific, targeted mutagenesis of the supF gene of the Lambda Phage genome from the purified DNA in an in vitro method (see specification, pages 14-18 and Table 1). The specification discloses that all except one of the 25 mutations produced by pso-AG10 is at or near the targeted T:A base pair at position 167 (see page 20). However, there is no evidence that said site-directed mutagenesis method would be operable in vivo. In example 5 of the specification, culture mouse fibroblast cells were site directed mutated by the oligonucleotide mutagen complex added to the growth medium and then UV irradiated in an ex vivo type method. However, there is no correlation between the entry of the oligonucleotide-mutagen complex in isolated cells in an ex vivo method and in vivo applications where entry into an animal is required.

There is a great deal of unpredictability in the modulation of nucleic acid interactions in vivo. Similar problems are also faced by ribozyme therapy. Uhlmann et al. (Chem. Reviews 90 : 544-584 (1990)) teach that the secondary and tertiary structure of the target nucleic acids have a critical influence on the efficiency of the target site and that it is impossible to predict the higher order structure of the mRNA and the effect it

will have on the efficacy of any potential inhibitory oligo (p. 576). Mirabelli et al (Anticancer Drug Design 6 :647-661 (1991)) teaches that we do not currently understand the precise role of nucleases, other intracellular enzymes and proteins on the stability of the ribozymes, the process by which oligonucleotides penetrate cellular membranes and distribute in cells, the non-sequence-specific-interactions that oligonucleotides can engage in both in and out of cells, and the metabolic pathways (both nuclease and non-nuclease) and metabolites that are likely to play a role in the metabolism of antisense drugs. Also undefined are the effects of specific base composition, length, chemical modifications of an oligo, and cellular parameters such as cell type, cell cycle phase and differentiation stage (Mirabelli et al, p. 651).

The post filing date art further confirms the unpredictability of this area. Puri et al (J. Biol. Chem. (2001) 276(31) :28991-28998) teaches "However, despite 40 years of research, there remain a number of impediments to the successful employment of TFOs as gene targeting reagents. Some of these obstacles reflect the properties of the oligonucleotides. Depending on the nature of the target either purine or pyrimidine TFOs can be used, but there are problems associated with each motif. Under physiological conditions purine TFOs are often subject to self structure formation which is incompatible with triplex formation. (see page 28991, column 2)". Thus, Puri expressly notes that years after Applicant's invention, the invention was still unpredictable. In fact, Puri finds that the nucleotides must be modified in a way not suggested by the application in order to achieve efficacy in what is an ex vivo assay. The complications involved in an in vivo assay would be significantly greater.

Lin et al (J. Biol. Chem. (2000) 275(50) :39117-39124) further supports the unpredictability of this art, noting that "We find that preformed triplexes on DNA that replicated following transfection are less stable than would be predicted by analyses of

triplexes in vitro or on total transfected DNA (page 39118, column 1)". The entire gist of the Li paper is that triplex formation ex vivo, in cells, is dramatically different and unpredictably different from triplex formation in vitro. These differences are magnified when compared to in vivo in animal experiments, where issues of delivery, penetration, and other similar issues become relevant.

Quantity of Experimentation

The quantity of experimentation in this area is extremely large since there is significant number of parameters which would have to be studied to apply this technology to in vivo methods, including the stability of the oligonucleotide-mutagen complex in blood and tissues, the distribution of oligonucleotides in tissues, the optimum mode of effective administration and the pharmacokinetics of administration. For an oligonucleotide mutagen complex, one must also consider (a) the ability of the oligonucleotide to specifically bind the target gene; (b) formation of a stable triple complex between the oligonucleotide and the target gene (note that modification of the oligonucleotide may interfere with its ability to form stable hydrogen bonds, etc.; (c) uptake of the oligonucleotide by the cell; (d) solubility of the oligonucleotide of the cell, and other such constraints. For example, with regard to the specificity issue, the AG10 oligonucleotide used in the specification is a subsequence and would hybridize to 54,417 sequences identified in Registry file. Even limiting this to humans yields 3,241 human sequences which comprise the AG10 sequence. The time table necessary to achieve efficacious administration of effective oligonucleotides, effective temperatures and pH conditions would require a very large quantity of experimentation for in vivo applications. This would require years of inventive effort, with each of the many

intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

Working Examples

The specification has no working examples of in vivo site directed mutagenesis using an oligonucleotide-mutagen complex. While there are in vitro and ex vivo examples, there are no in vivo working examples.

Guidance in the Specification.

The specification provides no evidence that the disclosed effective oligonucleotide-mutagen complexes would be able to modulate nucleic acid interactions or have usefulness in sequence specific triplex formation in vivo, let alone in humans or in a living animal or in plants. The guidance provided by the specification amounts to an invitation for the skilled artisan to try and follow the disclosed instructions to make and use the claimed invention. The specification merely discloses that if necessary for activation of the mutagen, light can be delivered to cells on the surface of the body, such as skin cells (see page 12). Even if, arguendo, the oligonucleotide-mutagen complex could enter skin cells in vivo (which the prior art and specification fail to enable), these claims are not limited to skin cells. There is no support for how cells in vivo could be activated by light as disclosed. The specification discloses that light can be delivered to cells within the body by fiber optic techniques or lasers by methods known to those skilled in the art (see page 12). However, a thorough review of the prior fails to show any enabled teachings of oligonucleotide-mutagen complex entering cells in vivo and being activated by light.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, in a highly unpredictable art where the oligomer-mutagen complexes effects in vivo depend upon numerous known and unknown parameters such as the metabolism specific to the target DNA, potential secondary structure, oligonucleotide length and oligonucleotide chemical composition for triplex DNA, the factor of unpredictability weighs heavily in favor of undue experimentation. Further, the prior art and the specification provides insufficient guidance to overcome the art recognized problems in the use of the oligonucleotide-mutagen complexes for in vivo treatment as broadly claimed (i.e encompassing a method in any cell under any treatment in any conditions). Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the absence of a working example and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

(11) Response to Argument

Introduction

The Board of Appeals and Interferences noted in the decision on the parent case, 08/083,088, that "In agreement with the examiner, we do not find that such evidence supports targeted gene therapy methods to any types of cells, targeting any type of target gene or protein, using the myriad and vast array of mutagens described in the specification having varying mutagenic potential and efficiency, which are encompassed by the broad scope of claim 6 (see Decision on Appeal, 08/083,088, page 10)". The current case has identical claims to those determined by the Board to be unpatentable under 35 U.S.C. 112, first paragraph. The Appellant begins with a discussion of the claimed invention. This discussion fails to appreciate the previous analysis by the Board as well as the rejection, both of which recognize that in vitro methods are enabled. The issue is that the broad claims read upon in vivo gene therapy in multicellular organisms and it is the breadth of the claims as they relate to in vivo gene therapy that lacks enablement.

New Evidence

Appellant then argues that the Declaration by Dr. Glazer, which represents the only new evidence in this application, supports the enablement of the claims because it demonstrates "substantial" uptake of triplex forming oligonucleotides (see Brief at page 6). The first two experiments were performed in vitro. Paragraph 12 of the Declaration details the only experiment in which multicellular organisms, specifically mice, were treated. The Declarant then discusses the results of experiments in which site specific

mutations are asserted to have been found in cells treated with TFOs which lacked a mutagen. However, this evidence is not drawn to claimed invention, in which a mutagen is required to be associated with the antisense oligonucleotide. Claim 6 requires that "the mutagenic oligonucleotide comprise a mutagen incorporated into a single-stranded nucleic acid (see claim 6)". The specification on pages 11 and 12 clearly indicates that the mutagen is something attached to the nucleic acid such as psoralen or an alkylating agent. The evidence presented does not include an oligonucleotide which falls within the scope of the claims. Consequently the evidence does not fall within the scope of the claim. Further, the oligonucleotides shown were not disclosed in the specification of the current claims, nor was the specific process of treatment taught by the specification. Thus, not only is the unpredictability of this invention supported by the art cited in the declaration, the declaration relies upon significant evidence which was not taught in the specification. Further, the presented evidence is not commensurate in scope with either the specification or the claims. The experiment in the Declaration uses a particular oligonucleotide and does not support the full scope of the claimed invention, which is drawn to mutagenesis of any site in any organism. Clearly, the limited polypurine sequence drawn to a arbitrary sequence inserted into a mouse does not support enablement over the full scope of the claims.

Even if a showing of isolated mutagenesis were made, this would not provide any use since no therapeutic effect has been shown for any of the oligonucleotides, even those asserted to induce mutagenesis. Simply correcting a few cells of the arbitrary mutation created in the mouse is not enough for a patentable use. The mutations must

be corrected in sufficient amounts to yield some benefit or there is no patentable use for the correction method. Forming, for example, 32 mutants out of 144,768 cells (see page 14 of declaration) would not appear to have any effect on the metabolism of the animal or any patentable use. Lastly, the declaration relies upon significant evidence which was not taught in the specification. Further, the oligonucleotides shown were not disclosed in the specification of the current claims, nor was the specific process of treatment taught by the specification.

Appellant then cites the legal standards regarding enablement and post-filing date art. The examiner agrees that later experimentation may validate a prophetic specification. However, the caselaw and MPEP 2164.05 requires that the "the experiments used the guidance in the specification as filed." Here, the experiments did not use the guidance in the specification as filed. The experiments left out a critical element required by the specification and by the claim, the mutagen. Without this critical element, the experiments do not provide evidence regarding the claimed invention, but rather show evidence regarding something else entirely. In fact, the Declaration itself also demonstrates that the evidence provided is **not the same as the method invented and claimed on June 24, 1993**. The Declaration cites Chan et al, who expressly makes this point, that the method of the Declaration and the method of the specification differ, stating "The tethered donor approach differs from our previous efforts to use triplex-targeted DNA damage to induce homologous recombination. In that work, the TFO was used to introduce site-specific psoralen photoadducts to stimulate recombination between two separate supF genes. In the work presented here, no

mutagen other than the triplex itself is involved, and the recombination is intended to occur not between two intact genes but between a target gene and a donor fragment tethered to the TFO. (page 11547, column 2)". So to the extent that the Chan reference demonstrates enablement of anything, it is a different method than that shown in the current specification which was neither taught nor suggested by the current specification. This same problem infects all of the other cited references because there is no discussion or evidence which demonstrates that these references operate by methodology taught in the current specification. As Chan notes regarding the efficacy of the tethered primer method "To our knowledge, this has not been reported previously (page 11547, column 2)". So Chan (on whose paper Dr. Glazer is a coauthor) expressly notes that as of 1999, it was not previously known that the tethered primer method without the use of a mutagen would function. Therefore, it is inappropriate and improper to rely upon the evidence of a different method, the site-directed mutagenesis using an oligonucleotide without an attached mutagen, to prove enablement of the claimed method, which is site-directed mutagenesis with an attached mutagen.

Appellant then argues the evidence regarding the monkey Cos cell and mouse fibroblast systems. These systems are in vitro, not in vivo systems, which do not fall within the scope of the rejection.

Teachings of the Art

While the art cited in the enablement rejection above demonstrates the unpredictability of the invention, even the art cited by Appellant does not support the enablement of the claims. In fact, the overwhelming teachings of the prior art cited by

Applicant oppose the enablement of the current claims. For example, Vasquez et al (Nucleic Acids Res. (1999) 27(4):1176-1181) states regarding the invention "However, if this approach is to be of practical utility in modifying a genome, then the mutation frequency at which this occurs must be increased (see page 1179, last sentence to page 1180)." This is an express admission that the method is not capable of function in vivo for any practical enabled use. Chan et al recognizes that the method, while perhaps a research tool in culture, is not yet ready for gene therapy, stating "Nonetheless, the TD-TFO approach as a method for DNA sequence modification has the potential to be a useful research tool and may eventually provide the basis of a gene therapy strategy. (see Chan et al, Journal of Biological Chemistry (1999) 274(17):11541-11548, see page 11548, column 2). Barre et al notes in the abstract of PNAS (2000) 97(7) that the efficiency, even in 2000, was too low for therapeutic applications in an in vitro setting, let alone an in vivo experiment, also stating "If this value is taken to represent the expected maximal frequency of mutations on an endogenous target, the proportion of targets reached by the TFO thus can be estimated as one in a thousand-a proportion too low to envision any therapeutic applications (see page 3088, column 1)." This statement also shows that the 32 of 144,768 mutations shown in the Declaration by Dr. Glazer, which is less than 1 in a thousand, is too low for any therapeutic application.

Dr. Glazer himself, the declarant, stated in an article in Science (1996) 271 that "On the other hand, the fact that triple helix formation can lead to mutations may be an important consideration in the use of oligonucleotides in research and as therapeutics.

Triplex-forming oligonucleotides designed to block transcription and even antisense oligonucleotides meant to prevent translation may have unintended and unexpected mutagenic effects." This is an express statement, by the Declarant, of the unpredictability of the invention. It is particularly striking since it is closer in time to the invention. In another paper, Dr. Glazer notes "Theoretically, targeted mutagenesis and inactivation of selected genes might also eventually have therapeutic applications. However, the general applicability of this approach will depend on the extension of the third-strand banding code and the development of nucleotide analogs so that triple helix formation is not limited to polypurine sequences. Much work in this regard is under way. Further experiments to develop a better understanding of cellular repair and replication of the triplex-directed lesion are also needed. In addition, the work reported here was performed with a highly constrained experimental model system, and targeted mutagenesis of a chromosomal gene by this approach has yet to be demonstrated. (see *Mol. Cell. Biol.* (1995) 15(3):1759-1768, page 1758)." This is an additional statement which indicates that undue experimentation would be required to apply the method in vivo. According to this statement, the method is not generally applicable, the work was in a highly constrained system, and no evidence that it functions on chromosomal genes exists. These statements support, and do not detract, from the enablement rejection. So to conclude the analysis of the art cited by Applicant, a final quote from a paper by Dr. Glazer regarding the goal of applying this method in vivo, "Development of novel base analogs and modifications to facilitate oligonucleotide binding, uptake, and resistance to hydrologic factors as well as research into new uses for the triplex

interaction will help move this technology from the benchtop to the bedside. Clearly, however, much more work needs to be done to achieve this goal. (see *Mol. Med.* (1997) 75:267-282, page 282)".

Appellant then argues that the claims were not separately considered. This argument is not correct since each and every claim is required to be enabled. The specific gene types in claims 11-14 do not affect whether the method will function in vivo. None of the specific agents listed in claims 8-10 are enabled for use in vivo. Therefore, the rejection of all the claims is proper since none of the specific claims is shown to affect the enablement of the invention in vivo.

Conclusion

Appellant is attempting to obtain a patent without providing the required roadmap and enabling disclosure. The rejection addresses each of the Wands factors and notes that the factors weigh heavily against finding enablement of the full scope of the claims. The previous decision by the Board recognized that analysis, stating "Thus, in view of the broad claim scope, unpredictability in the gene therapy art and mutagenesis art, the lack of working examples showing a therapeutic effect and showing absence of deleterious background or non-site specific target mutations using the method of the invention throughout the claim scope, lack of direction or guidance presented in the specification, and lack of evidence supporting enablement of the invention at the time of filing, we affirm the rejection under 35 U.S.C. 112, first paragraph of the examienr for lack of enablement (see pages 12-13 of Decision by BPAI, 08/083,088)." The current case differs from the parent case in that additional art

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was filed and a Declaration was filed with additional evidence. For the reasons given above, these elements do not overcome the prima facie case of nonenablement. The additional art actually supports the lack of enablement with express statements that show the unpredictability of the art even post filing. The Declaration does not overcome the rejection because it is not commensurate in scope with the claim and it fails to show therapeutic effect. As noted above, Barre stated that a value of 1 mutation in a thousand was too low to envision any therapeutic application. So the less than 1 mutation in 10,000 shown in the Declaration by Dr. Glazer, would also be too low for any therapeutic application.

For the above reasons, it is believed that the rejections should be sustained.

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JEFFREY FREDMAN
PRIMARY EXAMINER

Respectfully submitted,

Jeffrey Fredman
Primary Examiner
Art Unit 1634


August 6, 2003

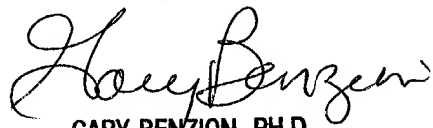
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